ORIGINAL ARTICLE

Biological Activities of Extracts Obtained From Natural Origin

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ABSTRACT

Background: Medicinal plants have been in use for curing ailments since hundreds of centuries. Extracts, both crude and purified are used in different forms and preparations to cure diseases. Various microorganisms have gained antibiotic resistance due to the overuse of drugs and widespread use of antibiotics.

Aim: To determine the antibacterial, antiproliferative and antioxidant activities of *Aloe vera medinensis*, *Azadirachta indica* and *Citrus aurantium*.

Methods: For this study, different parts of the selected medicinal plants were analyzed on various assays. These plants were collected from Gujranwala, Punjab and the extracts were prepared using the Cold maceration method in ethyl acetate and ethanol. The antibacterial activity was assessed against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus mirabilis* by disc diffusion method. The inhibitory zones were recorded in millimetres. The antiproliferative activity was assessed against HeLa cell lines using MTT assay. The absorbance was recorded at 570 nm. The antioxidant activity was assessed using Catalase and Superoxide dismutase assay. The absorbance was recorded at 240 nm for Catalase assay and 560 nm for Superoxide dismutase assay.

Results: The results indicate that commercially available antibiotics have better zones of inhibition against tested microorganisms. The antiproliferative activity was significant for the extract of neem leaves in ethanol and citrus fruit seeds in ethyl acetate. Antioxidant activity was found to be higher through SOD assay as compared to Catalase assay for most extracts. It can be concluded that the microorganisms were found to be susceptible to most of the extracts. Only two of the extracts showed antiproliferative activity whereas reasonably good antioxidant activity is shown by all of the extracts. These plants can be explored for further in depth analysis in animal models.

Keywords: Antimicrobial, antioxidant, antiproliferative, disc diffusion, MTT assay, Catalase assay

INTRODUCTION

Traditional therapies rely as much as 80% on the use of plant extracts or the active substances that have been derived from these extracts according to a report by World Health Organization, which involves the use of plant extracts or their active substances. Herbal plants are constituted of many secondary metabolites as essential oils that possess antibacterial and antifungal activities[8]. Active principles of these herbal extracts are also used as first ingredients for development of further drug chemistry modification[6,11,17,18].

Aloe vera is derived from an Arabic word 'alloeh' that means 'bitter'. Till date, approximately 275 species of *Aloe vera* have been documented[23]. Commonly known as Neem, *Azadirachta indica* is found in India and other Asian countries. This "Wonder plant" has been in use for the preparation of various medicines.

Salmonella typhi, Staphylococcus aureus and *Escherichia coli* have been found to be particularly susceptible to extracts of *Azadirachta indica* in vitro. Neem possesses significant antibacterial activity[21]. *Citrus aurantium* is quite rich in flavonoids and volatile oil. It also has a large portion of Vitamin C. Natural antioxidant activity of herbal plants is attributed to the presence of free radical scavengers that act as reducing agents and eliminators of excess oxygen. Galectin-3 stimulates neo-vascularization in vitro and in vivo, as well as acting as a chemo-attractant for the endothelial cells.

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MATERIALS & METHODS

It was a descriptive study which was conducted for a period of one year from June 2016-June 2017 in Microbiology laboratory of IMBB, The University of Lahore.

Samples of dry plant material including *Aloe vera*, Neem and Orange were collected from a local market, washed and rinsed with distilled water and then shade dried. The percentage of foreign matter was weighed in comparison to the whole weight of plant part used. Plant extracts were prepared by Cold maceration method. Whole or coarsely powdered crude plant material (about 200g) was placed in a stopper container with the solvent ethyl acetate and ethanol (600ml) and shaken routinely while kept at room temperature for about 3-7 days after which the preparation was passed through filter sheets to collect the filtrate. In total, twelve filtrates were obtained in solvents following which the solid or semi-solid plant extracts were stored in screw-capped bottles at room temperature till further use[22].

The scheme of extract preparation is given in Table 1. For the preparation of test concentrations, crude plant extracts were further dissolved in DMSO so that 500 and 1000µg/disk concentration was achieved. All extracts were applied against test organism in triplicate

Urine and blood samples were collected from 75 patients. Microorganisms were isolated through specific and different biochemical identification techniques and antimicrobial activity of different plant extracts was ascertained against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus mirabilis*. McFarland solution was used for adjusting the turbidity of inoculums for susceptibility testing. 0.5 ml of 1.175% (W/v) $BaCl_2.2H_2O$ was added in 99.5ml of 1% (v/v) H_2SO_4 to prepare the McFarland solution. The incubated bacterial strains were then suspended in sterile saline (0.89% NaCl) and turbidity was then matched to 0.5 McFarland solution for the

preparation of inoculums for disc diffusion assay.[22] Bacterial suspension (200µl) was poured and adsorbed over the surface of Muller-Hinton agar. Imipenem 5µg was used as positive control for *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. Amikacin 5µg was used as a positive control for *Klebsiella* and *Proteus*. The results were noted in the form of zone of inhibition (ZOI) in mm.

For antioxidant activity, SOD and Catalase assay were performed to determine the antioxidant activity of medicinal plant extracts. The absorbance was taken at 560 nm on an ELISA plate reader.

For the detection of antiproliferative activity of plant extracts, the samples were dissolved in DMSO at concentration of 20 mg/ml. The stock solutions were further diluted to concentration of 500µg in DMEM in a stock vial; these were filtrated by syringe filters measuring 0.22µm and later were stored in sterile aliquots in Falcon tubes at -20ºC until analyzed. HeLa cell lines which are human cervical cell lines were taken from cell culture laboratory of CRiMM (Center of Research in Molecular Medicine). DMEM-HG medium (Dulbecco's Modified Eagle's-High Glucose medium) that is supplemented with 10% heat inactivated (56ºC) fetal bovine serum, streptomycin (100mg/ ml) and Penicllin (100 IU/ml) was used to maintain the cervical cell lines.

MTT assay (3-(4,5-Dimethylthiazole-2-YI)-2,5-Diphenyltetrazolium Bromide assay) test was then applied to observe the effects of plant extracts on surviving potential of cancerous cells. Viable cell number in each of the wells was proportionally taken to be equal to the intensity of absorbance of light and the measurement of this absorbance was then read on an ELISA plate reader at 570 nm.

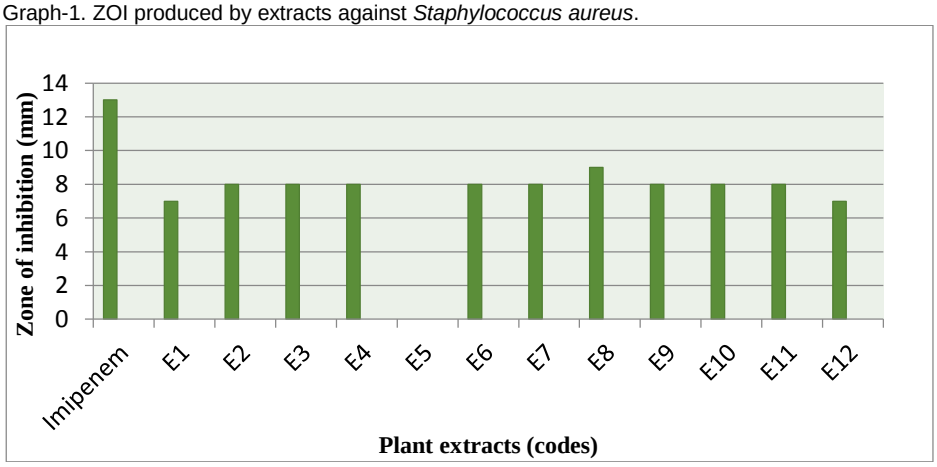
Data obtained through MTT assay, SOD and Catalase assays were statistically analyzed by Graph pad prism. Values at $P \leq 0.05$ were considered to be statistically significant.

RESULTS

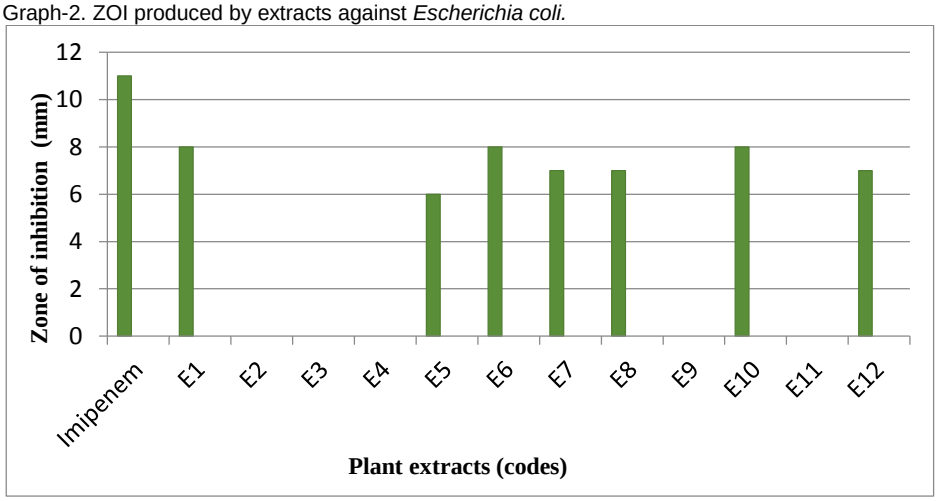
Table 1: Preparation of extracts in respective solvents

S.No	Extract No.	Preparation of extract
1	Extract 1-E1	Ethyl acetate+ *Aloe vera* leaf cuticl
2	Extract 2-E2	Ethanol+ *Aloe vera* leaf cuticle
3	Extract 3-E3	Ethyl acetate+ *Aloe vera* gel
4	Extract 4-E4	Ethanol+ *Aloe vera* gel
5	Extract 5-E5	Ethyl acetate +Neem leaves
6	Extract 6-E6	Ethanol+ Neem leaves
7	Extract 7-E7	Ethyl acetate+ Neem stems
8	Extract 8-E8	Ethanol + Neem stems
9	Extract 9-E9	Ethyl acetate +Citrus fruit peel
10	Extract 10-E10	Ethanol +Citrus fruit peel
11	Extract 11-E11	Ethyl acetate+ Citrus fruit seeds
12	Extract 12-E12	Extract 12-E12

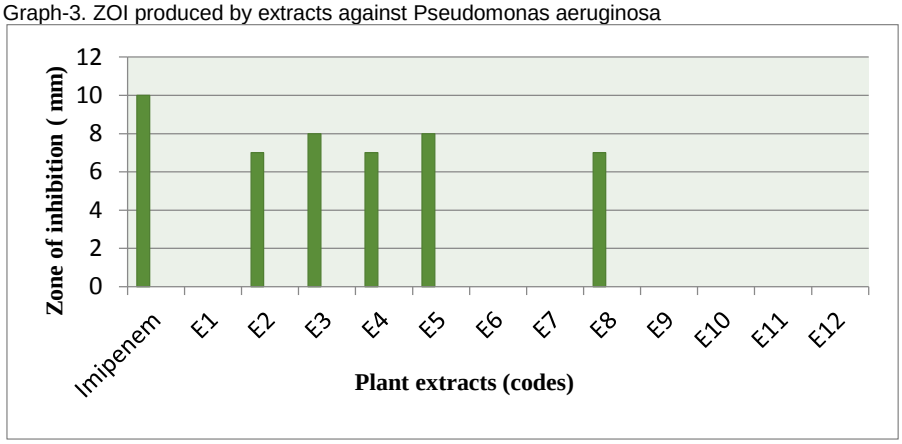
Graph-1. ZOI produced by extracts against *Staphylococcus aureus*.



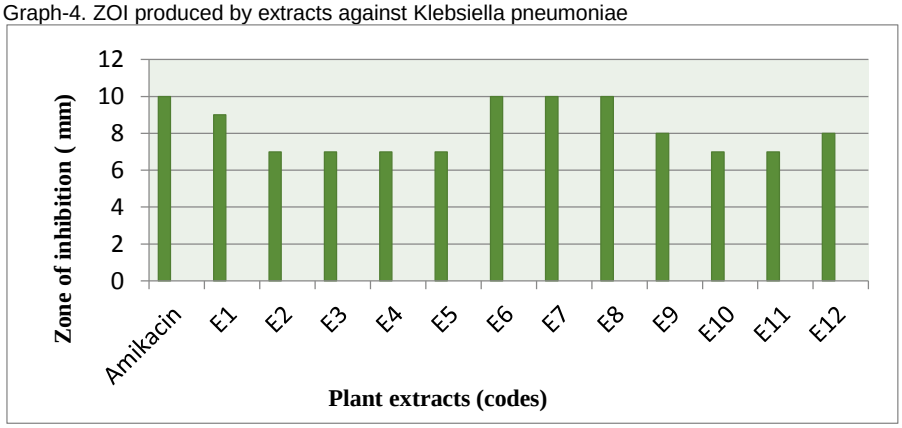
Graph-2. ZOI produced by extracts against *Escherichia coli.*



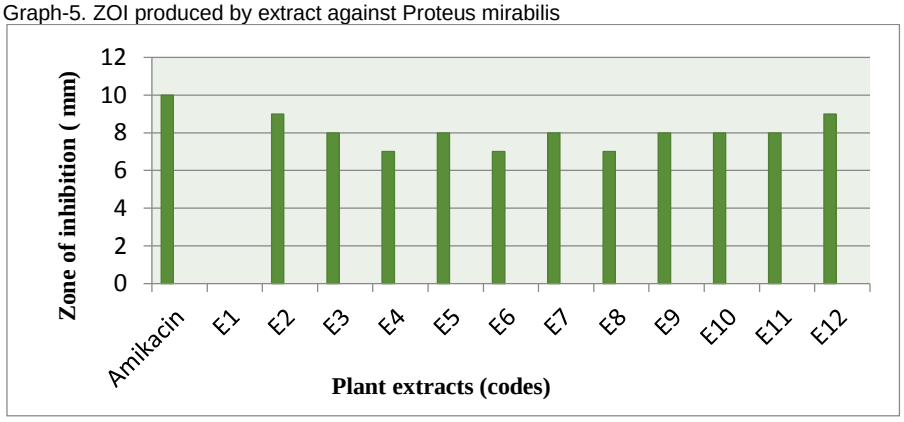
Graph-3. ZOI produced by extracts against Pseudomonas aeruginosa



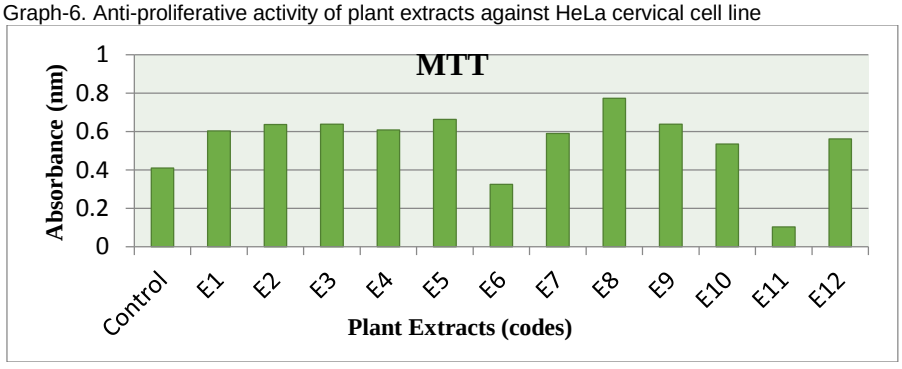
Graph-4. ZOI produced by extracts against Klebsiella pneumoniae



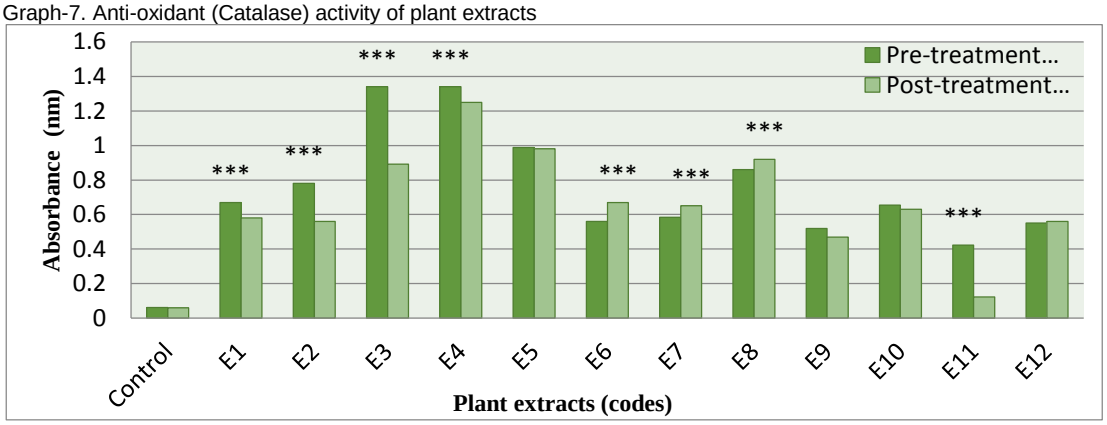
Graph-5. ZOI produced by extract against Proteus mirabilis



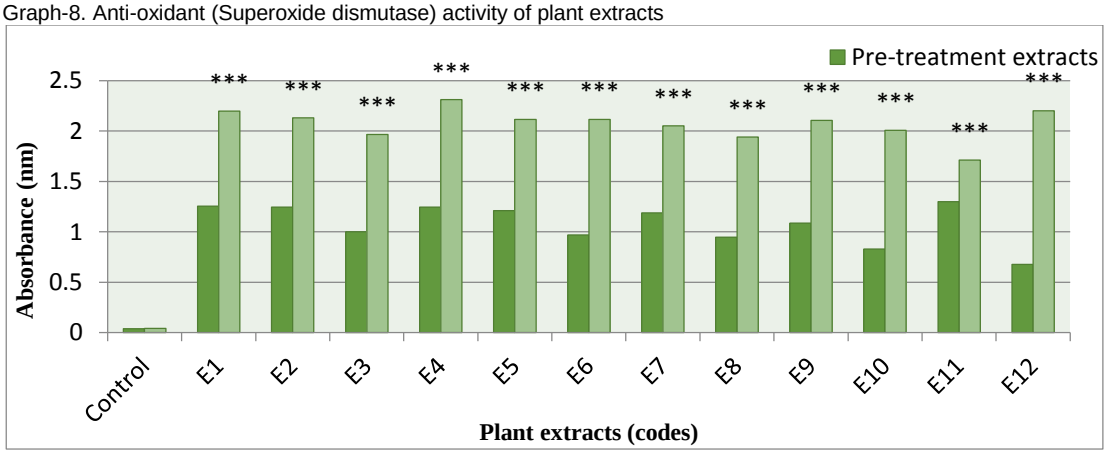
Graph-6. Anti-proliferative activity of plant extracts against HeLa cervical cell line



Graph-7. Anti-oxidant (Catalase) activity of plant extracts



Graph-8. Anti-oxidant (Superoxide dismutase) activity of plant extracts



DISCUSSION

Aloe vera leaf cuticle in both ethyl acetate and ethanol exhibited activity against *Staphylococcus aureus.* Reports by Abdullah KM *et al.,* 2003 and Asma *et al.,* 2007 also complement this finding. *Aloe vera* gel in both ethanol and ethyl acetate show antibacterial activity against *S. aureus.* Leaf extracts in ethyl alcohol showed activity against *E.coli* revealing that *Aloe vera* has antimicrobial action against both gram positive and gram negative bacteria. *Aloe vera* gel and leaf extracts have activity against *Klebsiella pneumoniae* but only the leaf cuticle extract in ethanol showed activity against *Pseudomonas.* Extracts of *Azadirachta indica* leaves and stem showed more activity against *E. coli* and *K. pneumoniae* as compared to *S. aureus* depicting thereby that Neem plant is more active against gram negative bacteria. The antimicrobial potential of Neem according to this study is similar to reports of Biswas *et al.,* 2003. Neem leaves in ethyl acetate showed no activity against *S. aureus.* More antimicrobial activity was exhibited by Neem extracts against *E. coli, K. pneumoniae* and *P. mirabilis.* Neem extracts in ethyl acetate and ethanol exhibited reduced activity against *Pseudomonas.* This finding is contrary to reports of Kusum *et al.,* 2013 which states significant activity of Neem against *P. aeruginosa.* Perhaps the difference of finding between this study and reports by Kusum *et al.,* 2013 is due to the different geographic locales whereby the plant has been collected from extracts derived from *Citrus aurantium* fruit peel and seeds in ethyl acetate and ethanol showed antimicrobial activity against *S. aureus, Proteus* and *Klebsiella.* Greater activity was exhibited by *Citrus aurantium* fruit seeds in ethanol against *Proteus mirabilis* and *Klebsiella pneumoniae.* Activity against *Escherichia coli* was only shown by *Citrus aurantium* fruit peel in ethanol and *Citrus aurantium* fruit seeds in ethanol. This finding is complemented by reports of Arias BA *et al.,* 2005. Pharmacological actions for *C. aurantium* include being an anti-inflammatory and an antibacterial agent.[10] However, *Citrus aurantium* seeds and fruit peel extract in ethanol and ethyl acetate showed no antimicrobial activity against *Pseudomonas aeruginosa* showing that *Pseudomonas* is not acted upon by any of the compounds found in *Citrus aurantium. Citrus aurantium* therefore shows antimicrobial activity against both gram positive and gram negative bacteria.

Azadirachta indica leaves in ethanol and *Citrus aurantium* fruit seeds in ethyl acetate have antiproliferative action. Both of these extracts had significant reducing effect on HeLa cervical cell lines. However, *Aloe vera* extracts showed no antiproliferative activity and reducing effect on HeLa cell lines. This is contrary to reports presented by Furukawa *et al.,* 2002 and Abdullah KM *et al.,* 2003. *Citrus aurantium* has a unique triterpenoid that possesses chemopreventive properties that are active against human colon carcinoma cells.[14,15] The results of this study complement the results of Richardson J *et al.,* 2005.

The antioxidant enzymes are superoxide dismutase, catalase and glutathione peroxidise.[16] The results of this study show that extracts of *Aloe vera* possess antioxidant potential. Both leaf cuticle and *Aloe vera* gel have antioxidant activity.
Thus, the results of this study are complemented by reports presented by Langmead *et al.,* 2004 and Gallagher and Gray *et al.,* 2003. The extracts of *Azadirachta indica* show

antioxidant properties and this is complemented by reports presented by Bandyopadhyay *et al.,*2002 and Amal Kumar Ghimeray *et al.,* 2009. The work on *Citrus aurantium* has shown significant antioxidant activity. Extracts of *Citrus aurantium* exhibit increased superoxide dismutase activity.

CONCLUSION

The results of this study state that the extracts of *Aloe vera medinensis, Azadirachta indica* and *Citrus aurantium* exhibit reasonable bactericidal activity as depicted from disc diffusion method and if the doses of these extracts are increased within therapeutic limits, a greater bactericidal activity can be hoped for. Thus, these plants hold promise as better alternatives to conventional synthetic medicine with fewer side effects advantageously.

Azadirachta indica leaves and *Citrus aurantium* seeds hold potential as antiproliferative agents as shown by MTT assay. They can be further studied upon with in depth techniques and more detailed studies for pathway analysis. Antioxidant activity is significant in various extracts with SOD activity more pronounced as shown in results. Hence these plant parts can be further processed and formulated for the benefit of medicinal field.

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